Effects of hepatic portal infusion of deionized water on metabolic and hormonal responses to exercise in rats

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Latour, Martin G., François Désy, Claude Warren, and Jean-Marc Lavoie. Effects of hepatic portal infusion of deionized water on metabolic and hormonal responses to exercise in rats. J. Appl. Physiol. 84(5): 1653–1660, 1998.—The present study was conducted to investigate the in vivo effects of an intrahepatic infusion of deionized water during exercise in rats. Adrenomedullated male Sprague-Dawley rats were continuously infused for 30 min either at rest or during treadmill exercise (26 m/min, 0% grade). Rats were randomly assigned to one of three infusion conditions (52 μl/min) with either deionized water (PW) or saline (PS; NaCl; 0.9%) via the hepatic portal vein or deionized water through the jugular vein (JW). The exercise period caused a significant (P < 0.05) decrease in liver glycogen and relative liver water content and peripheral and portal blood glucose and insulin while increasing peripheral and portal glucagon and K⁺ plasma concentrations. These responses, with the exception of K⁺, were not influenced by the different types of infusions. The increase in K⁺ during exercise was significantly (P < 0.05) higher in JW rats than in the PW and PS groups. Both the infusion and exercise protocols did not significantly alter the liver weight-to-body weight ratio, plasma osmolality, free fatty acids, β-hydroxybutyrate, Na⁺, Cl⁻, vasopressin, and catecholamine concentrations. It is concluded that an hepatic portal infusion of deionized water does not specifically alter the metabolic and hormonal responses to exercise in rats.

portal receptors; insulin; glucagon; catecholamines

PUTATIVE HEPATIC RECEPTORS have been reported to play a regulatory role in different physiological situations, such as control of food intake (24, 29), insulin-induced hypoglycemia (8, 16), and physical exercise (5, 17). Concomitantly, several studies using different approaches have shown that these hepatic receptors can modify the insulin (19, 26), glucagon (17), and plasma catecholamine (8, 16) responses. The best evidence in favor of such a role by the liver during exercise comes from the demonstration that hepatic vagotomy attenuates the exercise-induced reduction in insulin and increase in glucagon in adrenomedullated rats (17). Although the existence and physiological action of the hepatic receptors are experimentally well substantiated, the nature of the metabolic activity in the liver and the regulatory mechanism responsible for this afferent activity are poorly understood. Different substrates such as glucose (21, 22), pyruvate (5, 7), and different amino acids (27, 28) have been postulated to be at the origin of the hepatic afferent information.

In the present study, we hypothesized that deionized water is a substance that may influence the hepatic afferent information of exercise. The interest in studying the effects of deionized water infused into the hepatic portal circulation during exercise comes from two postulated physiological regulatory mechanisms: the existence of hepatoportal water-sensitive receptors and the possibility of alterations of hepatic cell volume. Hepatic portal infusion of water has been reported to suppress water intake in water-deprived rats (13), which was not observed in hepatic vagotomized rats (14). These data suggest an hypotonic stimulation of hepatoportal osmoreceptors. It has also been reported that splanchnic osmosensors signaling hyposmolality are mediated through hepatic vagal afferents (2). On the basis of anatomic studies (4), these authors (2) suggested that the water-sensitive receptors may be located in the portal vein area. Because the hepatic branch of the vagus nerve has been shown to be involved in the exercise-induced hormonal response (17), it is possible that a hypotonic stimulation of the hepatoportal osmoreceptors contributes or interferes with the hepatic afferent information.

The interest in studying hypotonic infusion of water into the hepatoportal area during exercise also stems from the recent reports that hepatic metabolism appears to be regulated by a new parameter, i.e., cell volume (10, 11). In the liver, it seems that alterations of cell volume markedly influence a variety of metabolic pathways, such as protein and carbohydrate metabolism, not primarily serving cell volume regulation (11). In perfused liver, insulin, by acting on different transport systems, leads to cellular accumulation of K⁺, Na⁺, and Cl⁻ and, consequently, to cell swelling (12). Glucagon, on the other hand, is known to decrease cellular K⁺ in isolated perfused rat liver, resulting in cell shrinkage (9). This is of interest for the physical exercise situation because, during exercise, insulin concentration decreases and glucagon concentration increases. Both of these stimuli should lead to a shrinking of liver cells. Although the present study was not designed to study hepatic cell volume alterations during exercise, it is possible that an intraportal hypotonic infusion of water may alter the endocrine response to exercise by altering hepatic cell volume regulation. The purpose of the present experiment was, therefore, to specifically study the effects of an hepatic portal infusion of largely hypotonic water (deionized) on the metabolic and hormonal responses to exercise in adrenomedullated rats.

METHODS

Animal care. Male Sprague-Dawley strain rats (Charles River Canada, St.-Constant, Québec), weighing 180–200 g, were housed in individual cages and allowed pellet rat chow and tap water ad libitum for 25 days after they were received in our laboratory. Lights were on from 0700 until 1900, and
the room temperature was maintained at 20–23°C. Three days after their arrival, all rats were surgically adrenomedullated and allowed to recover for 3 wk to permit adrenal cortical regeneration. This was done, as in some of our previous studies (5, 6), to avoid the inhibitory effect of epinephrine on insulin secretion. During this time, rats underwent a running-habituation protocol, consisting of 10 sessions over 2 wk, beginning with 15 min/day at 15 m/min and progressively increasing to 55 min/day at 30 m/min (0% grade) on a motor-driven rodent treadmill.

Surgery. Five days before the experiment, rats underwent a jugular and a hepatic portal vein cannulation under pentobarbital sodium (40 mg/kg ip) anesthesia. The jugular catheter was implanted by a method previously described (18). The hepatic portal catheter was inserted according to the technique described by Tordoff et al. (30) with some minor modifications (5). Briefly, the catheter consisted of a 20-cm-long Silastic tube (0.51 mm ID, 0.94 mm OD, no. 602–135, Dow Corning) protected at the distal end by a 7-cm-long Tygon microbore tubing sheath (ID 1.02 mm ID, 1.18 OD, no. S-54-HL). First, the catheter was tunneled subcutaneously from the abdominal laparotomy to the back of the neck of the animal. At that point, the catheter was attached to the skin with the help of a small piece of tulle mesh that had been previously attached to the distal portion of the catheter. The external end of the catheter was also made of a 23-gauge cut needle and 3-cm polyethylene tubing (PE-50, 0.965 mm ID, 0.965 mm OD, no. 427411, Clay Adams). The internal end of the catheter was beveled at −45°. Once the catheter was positioned in the portal vein, the cecum was retracted from the abdominal cavity and the ileocolic vein was located. The entry point of the catheter was determined at the intersection of two tributaries of the ileocolic vein. The catheter was then threaded toward the portal vein and tied at the insertion point with previously placed sutures. A verification of placement of the cannula was made postmortem to ensure the success of the portal infusion.

Group and exercise protocol. The night before experimentation, all rats received only 50% of their daily food intake [10.4 ± 1.1 (SE) g]. Feeding was done in this way to reduce liver glycogen concentrations and to better stimulate the counterregulatory response during exercise. On the day of the experiment, rats were divided into a resting group and an exercising group. Both groups were further divided into three subgroups. Two subgroups received a hepatic portal infusion of either deionized water (PW; HPLC grade, Millipore) or sterile saline (PS; NaCl, 0.9%). The third subgroup (JW) received a jugular infusion of deionized water. The infusions were made with the use of a microinfusion pump at a rate of 52 µl/min (Harvard Apparatus). This rate of water infusion is the one used by Kobaski and Adachi (13, 14), who found that such an hepatic portal infusion of water suppresses water intake in water-deprived rats. The morning of the experiment, rats were weighed and the catheters were connected with an extension (PE-50). A 15-min rest was then allowed before the beginning of the infusion protocol at rest or during exercise. The experiment was run between 0800 and 1100. The exercise test consisted of the rats running on the treadmill at 26 m/min (0% grade) while being continuously infused for 30 min. Resting groups were also infused during 30 min in their individual cage. At the end of the exercise, rats were rapidly anesthetized via the venous catheter with pentobarbital sodium (20 mg/kg) while still running. Immediately, the abdominal cavity was opened and ~5 and 3 ml of blood were simultaneously collected via the abdominal vena cava and the portal vein, respectively (~45 s). Immediately afterward, a small piece of liver was taken from the frontal lobe, frozen with aluminum block cooled to liquid-nitrogen temperature. Afterward, the liver was excised, cleaned of extrahepatic tissues, and patted dry before being weighed and then frozen in liquid nitrogen. Nonexercised rats were treated in the same manner as the exercised rats and were killed at approximately the same time.

Analytic methods. Peripheral blood was collected into 5-ml syringes with 7% EDTA and immediately separated into four fractions. A small portion of blood was used for hematocrit determination in triplicate by using the microhematocrit method and corrected for trapped plasma (31). The second fraction of blood (500 µl) was preserved in trasylol (50 µl) and
centrifuged, and the plasma was used for glucagon determination. The third fraction of blood (1.5 ml) to be used for catecholamine determination was transferred in tubes containing 50 µl of glutathione (60 mg/ml) and EGTA (90 mg/ml), kept in crushed ice, and centrifuged (4°C at 2,500 rpm, table Beckman GPR centrifuge) within 30 min after collection. The remainder of the blood was also centrifuged (Eppendorf centrifuge, no. 5415), and the plasma was stored for subsequent glucose, insulin, lactate, free fatty acids, β-hydroxybutyrate, osmolality, and electrolyte (Na⁺, K⁺, and Cl⁻) determinations. Portal blood was also collected into syringes with EDTA and treated similarly for osmolality, hematocrit, electrolytes, glucagon, and insulin determinations. All tissue and blood samples were stored at −78°C until analyses were performed.

Plasma glucose and lactate concentrations were determined by the use of a glucose-lactate analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Insulin and glucagon concentrations were determined by commercially available radioimmunoassay kits (Radioassay System Laboratory; ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montreal, Quebec). Free fatty acids and β-hydroxybutyrate were assessed enzymatically with the use of reagent kits from Bohringer Mannheim Laboratories (distributed by Immunocorp). Vasopressin concentrations were determined with a radioimmunoassay kit available from Buhlmann Laboratories. Catecholamines were extracted from plasma according to the procedure described by Rémy and Zaagsma (23) and quantified by means of an isocratic HPLC system (Waters Division, Millipore). The recovery of norepinephrine, epinephrine, and dihydroxybenzylamine at a concentration of 2 ng/ml was 95.8 ± 8.4, 94.5 ± 4.6, and 79.1 ± 4.3%, respectively. Plasma electrolytes (Na⁺, K⁺, and Cl⁻) were analyzed by use of automatic analyzer no. 704 from Bohringer Mannheim/Hitachi. The plasma osmolality was physically determined by use of an osmometer (Advanced Instrument Microsrometer, model 3Mo). The liver was precisely weighed with an electronic balance (Mettler AE 100), and its glycogen concentration was determined by use of the phenol-sulfuric acid reaction (20). Liver dry weight was determined after the liver was freeze-dried while being kept frozen at −70°C. The percentage of water content of all livers was determined by computing the ratio of the liver dry weight to the liver wet weight. Liver glycogen content was computed as the product of liver glycogen concentrations and liver wet weight. All data are reported as means ± SE. Statistical analyses were performed by a two-way analysis of variance non-repeated

Fig. 2. Plasma glucose (A), lactate (B), free fatty acids (C), and β-hydroxybutyrate concentrations (D) in rats at rest and after exercise. Values are means ± SE; n = 5–10 rats at each point. Significantly different from corresponding resting values, **P < 0.01. Significantly different from JW group, * P < 0.05.
measures design. Tukey’s post hoc test was used in the event of a significant ($P < 0.05$) F-ratio.

**RESULTS**

The ratio of liver weight to 100 g body weight was not changed significantly by the infusion or by the exercise stimulus (Fig. 1A). However, the liver water content significantly ($P < 0.05$) decreased with exercise in all groups (Fig. 1B). A tendency ($P < 0.08$) for the liver water content at rest to be higher in the PW than in the PS group was observed. As expected, liver glycogen content was significantly ($P < 0.01$) decreased during exercise in all groups (Fig. 1C). This decrease was not affected by the infusions. Blood glucose concentrations were decreased significantly ($P < 0.05$) and similarly in all groups during exercise (Fig. 2A). Blood lactate and $\beta$-hydroxybutyrate concentrations were not significantly affected by the exercise or by the infusion stimulus (Fig. 2B and D, respectively). Free fatty acid concentrations were not changed with exercise in all groups, although a significant ($P < 0.05$) difference in resting levels was found between PS and JW groups (Fig. 2C).

Peripheral and portal plasma insulin and glucagon concentrations were significantly ($P < 0.05$) decreased and increased, respectively, during exercise in all groups (Fig. 3). These responses were not affected by the different infusions. Both epinephrine and norepinephrine concentrations were not affected by the exercise or infusion protocols (Fig. 4, A and B, respectively), although a tendency ($P < 0.07$) for norepinephrine to be increased in all groups at the end of the exercise period was found. Because all rats were adrenodemedullated, low concentrations of epinephrine were found at rest as well as after exercise (Fig. 4A).

Plasma osmolality, hematocrit, and vasopressin values were not significantly changed by either water infusion or exercise (Fig. 5). A tendency ($P < 0.07$) for portal hematocrit to decrease with exercise was measured (Fig. 5D). All measured electrolytes, either in peripheral or portal circulation, were not affected by the water infusions (Fig. 6). Exercise, however, resulted
in a significant \((P < 0.05)\) increase in both peripheral and portal \(K^+\) concentrations (Fig. 6, C and D). The increase in portal \(K^+\) concentrations with exercise was significantly \((P < 0.05)\) more pronounced in the JW group than in PS and PW groups (Fig. 6D).

**DISCUSSION**

In this study, deionized water was used as a hypotonic stimulus in the hepatic portal area in vivo to evaluate the possibility of altering the hormonal response during exercise. Hepatic portal infusion of water has already been reported to suppress water intake in water-deprived rats (13, 14), suggesting the existence of hypotonic osmoreceptors in the hepatoportal area. On the basis of this information, we hypothesized that an intraportal infusion of deionized water during an exercise session could alter hepatic afferent signals and, by so doing, modify the dependent endocrine and metabolic responses. With the idea that an intraportal infusion of deionized water can alter hormonal and metabolic responses, one must keep in mind that the liver is equipped with nerves and sensitive afferent fibers that play a substantial role in detecting the incoming flow of different nutrients and metabolites (22, 25). Our interest was mainly directed to the pancreatic hormone and the norepinephrine responses. These hormone responses to exercise have been modified in the past after chronic hepatic vagotomy (6) and intraportal infusion of pyruvate (5). Results of the present study, however, show that none of these hormonal responses to exercise was modified by an intraportal infusion of deionized water compared with the same infusion in the jugular vein and compared with an intraportal infusion of isotonic saline solution. These data, therefore, do not support the hypothesis that the hypotonic osmoreceptors in the hepatoportal area may in some way contribute to the hormonal response to exercise.

Besides a possible contribution of the hepatic hypotonic osmoreceptors during exercise, the intraportal infusion of deionized water was also used in the present study in an attempt to counteract a possible shrinking effect of exercise on liver cells. Although liver cell volume was not measured in the present experiment, it is interesting to note that the intraportal infusion of deionized water resulted in a statistical tendency \((P < 0.08)\) toward a higher liver water content at rest in this group of rats than in the two other conditions (Fig. 1B). It is also noteworthy to observe that liver water content was decreased during exercise. Although this decrease seems to be more pronounced in the PW group, the statistical analysis did not show any discrimination among groups. Given the limitations of the technique used to measure liver water content, the present results suggest that the liver cells might have lost water during exercise. This is supported by the recent demonstration from our laboratory (16), using the multiple-indicator dilution curve technique, of a 15% decrease in hepatocyte volume in rats after 60 min of exercise. The shrinking of hepatocytes by itself may be able to stimulate the afferent nervous pathway (hepatic vagus nerve) and in this way influence the metabolic regulation of exercise. It was postulated that an intraportal infusion of deionized water may counteract this effect and in this way reduce, as would an hepatic vagotomy (6, 17), the hormonal response to exercise. The present data, however, do not provide any evidence that such a mechanism is operative during exercise.

Osmolality, as well as sodium concentration, was not affected differently by the different infusions of water or by the exercise stimulus. Volume expansion, as reflected by the hematocrit values, was not affected by the different infusions, either. This suggests that the animals probably reacted to the different water infusions by adjusting urine output at rest as well as during exercise. Although urine output was not measured in the present experiment, observations made during the experiment indicate that urine output was largely increased. No differences in vasopressin response among the groups were observed at rest and after exercise. This response is probably linked to the osmolality response. In behavioral studies, portal vein infusion of hypotonic solution has been reported to reduce water intake in water-deprived rats (13). This suppression of drinking was abolished by hepatic vagotomy (14). He-
Patic vagotomy or total liver denervation does not, however, affect water intake in freely fed and watered rats (1, 3). It is possible that the present portal infusion of deionized water might have caused some changes in the metabolic and hormonal responses to exercise if the animals had been previously water deprived. This was not done in the present experiment, however, because our purpose was to test the possibility that hepatic osmosensors may contribute to exercise regulation in a natural situation and to avoid reduction in plasma volume that, in turn, affects the normal response to exercise. Overall, the present results suggest that the animals adjusted their blood volume in a similar way to the different types and sites of water infusion, at rest as well as during exercise. In addition to the hormonal responses, the metabolic responses to exercise were similar in all three groups of rats. As expected, liver glycogen contents reached low levels in all groups, resulting in a similar decrease in blood glucose levels. The lack of effects of exercise on free fatty acids and β-hydroxybutyrate may be due to the relatively short duration of exercise (30 min). The increase in plasma K⁺ concentrations during exercise is well documented (31). The larger portal K⁺ concentration in the JW

Fig. 5. Peripheral and portal plasma osmolality (A and B, respectively) and hematocrit (C and D, respectively) and vasopressin (E) at rest and after exercise. Values are means ± SE; n = 5–10 rats at each point.
group compared with in the two portal-infused groups (Fig. 6D) might be due to a dilution effect of the portal infusion.

In summary, results of the present experiment indicate that an intraportal infusion of deionized water, compared with an intraportal infusion of saline or a jugular infusion of deionized water, does not influence the metabolic and hormonal responses to a 30-min exercise bout in adrenodemedullated rats. These results do not provide any evidence that the hepatic hypotonic osmosensors reported to increase water intake (13, 14) contribute to the hormonal response to exercise.

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